method for the analysis of 1,3-butadiene in whole blood from mice and rats (Himmelstein et al. 1994).

Briefly, 200 µL of whole blood, obtained by cardiac puncture, was transferred into pre-labeled, capped, and weighed airtight headspace vials (1.5 mL autosampler vial). Sample vials were weighed to obtain an accurate estimate of sample size and allowed to equilibrate at room temperature for 2 hours. Once equilibration was complete, samples were analyzed using an Agilent 5973 mass spectrometer coupled to an Agilent 6890 gas chromatograph. The mass spectrum was run in electron impact mode with selective ion monitoring (instrumental conditions are listed below).

Calibration curves were prepared by spiking stock control whole blood with known amounts of chloroprene obtained as a certified standard solution of chloroprene in methanol. Quality control samples were prepared by spiking control rat plasma with a certified chloroprenestandard. QC samples were spiked to low (near the first calibration point), medium (near the middle of the calibration curve), and high (near the highest point of the calibration curve) levels. Aliquots of the prepared QC's were placed in sealed GC vials (3 aliquots for each level, 9 total) and kept frozen at -80 °C until required (GC vials had a minimum of headspace prior to freezing). On the blood collection days, a low-, middle-, and high-level QC was thawed and allowed to come to room temperature for 4 hours. After this time, the QC samples were "sampled" with a syringe identical to those being used for the collection of whole blood, placed in a GC vial in a manner identical to that of the whole blood collection, and analyzed along with the samples and standards.

Additional details of the nose-only inhalation study can be found in IISRP (2009a), which is provided in Supplemental Materials A.

Chloroprene PBPK Model

Model Structure

The structure of the PBPK model used in this study (Figure 1) is based on the PBPK model of chloroprene described in Himmelstein et al. (2004b), as modified by Yang et al. (2012). As in previous models of volatile organic compounds (Ramsey and Andersen 1984, Andersen et al.

1987), the blood is described using a steady-state approximation and the model assumes blood-flow limited transport to tissues and venous equilibration of tissues with the blood. Metabolism is described in the liver, lung and kidney using Michaelis-Menten saturable kinetics.

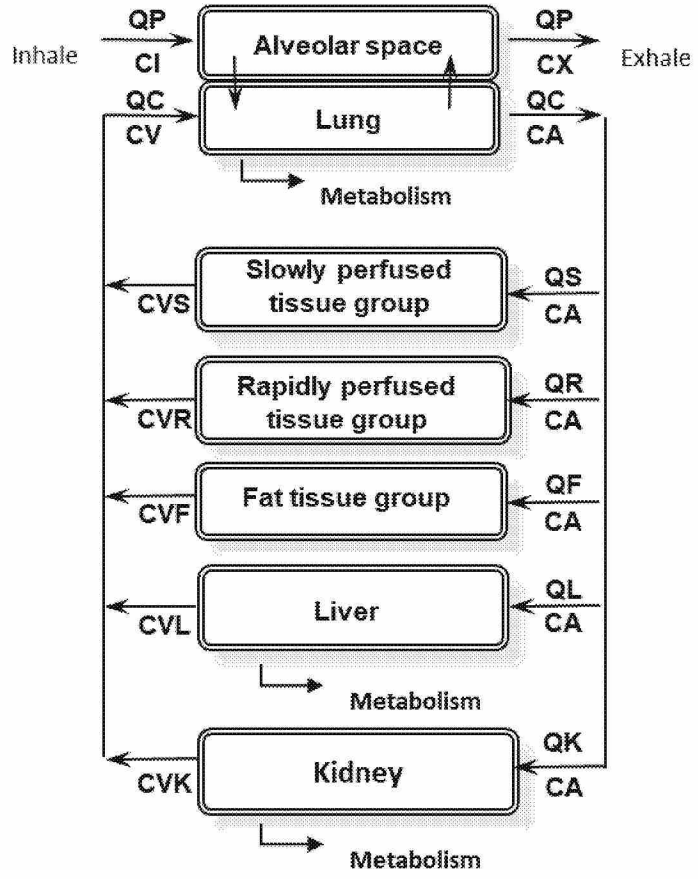

Figure 1: Chloroprene PBPK model diagram. Abbreviations: QP - alveolar ventilation; CI - inhaled concentration; CX - exhaled concentration; QC - cardiac output;CA - arterial blood concentration; CV - venous blood concentration; QS, CVS - blood flow to, and venous concentration leaving, the slowly perfused tissues (e.g., muscle); QR, CVR - blood flow to, and venous concentration leaving, the richly perfused tissues (most organs); QF, CVF - blood flow to, and venous concentration leaving, the fat; QL, CVL - blood flow to, and venous concentration leaving, the liver; QK, CVK - blood flow to, and venous concentration leaving, the kidney.

Model Parameters

All physiological parameters in the model for mouse, rat and human (Table S-1 in Supplemental Materials B) are taken from Brown et al. (1997) except for the cardiac output in the mouse and the alveolar ventilation and cardiac output in the human. While the alveolar ventilation in the mouse is taken from Brown et al. (1997), relying on the value of cardiac output reported in Brown et al. (1997) would result in a value of 11.6 L/hr/$bw^{3/4}$ for cardiac output (QCC). If used with the Brown et al . (1997) value of 29.1 L/hr/$bw^{3/4}$ for alveolar ventilation (QPC), this would result in a serious mismatch between ventilation and perfusion (V/Q ratio >> 1). Andersen et al. (1987), the developers of the PBPK model for methylene chloride that was used in the USEPA (2011) IRIS assessment, argued that it would be more biologically realistic to assume that the V/Q ratio was close to 1 at rest, and stated that their previous experience with PBPK modeling of data on clearance of chemicals in the mouse under flow-limited metabolism conditions supported the use of a higher value for QCC. Therefore, the value of QCC in the current model was calculated by dividing the alveolar ventilation from Brown et al. (1997) by MCMC estimates of a V/Q ratio for the mouse based on pharmacokinetic data for exposures to another volatile organic chemical, methylene chloride (Marino et al. 2006), which was used in the USEPA (2011) inhalation cancer risk assessment for that chemical. In the case of the human, it is more appropriate to use the default EPA ventilation rate of 20 L/day, reflecting an average activity level, rather than a resting value (Clewell et al. 2001). Since the values for alveolar ventilation and cardiac output in Brown et al. (1997) are resting values, we used the values calculated for the PBPK model of vinyl chloride (Clewell et al. 2001), which was developed for the USEPA (2000) cancer risk assessment for that chemical.

Apart from the physiological parameters, the model parameters are based entirely on *in vitro* data. The partition coefficients (Table S-2 in Supplemental Materials B) were calculated from the results of *in vitro* vial equilibration data reported by Himmelstein et al. (2004b), using the partition coefficients for muscle and kidney to represent the slowly and rapidly perfused tissues, respectively. To obtain the model parameters for metabolism in the liver, lung and kidney, the original *in vitro* chloroprene metabolism time-course data (Himmelstein et al. 2004a, IISRP 2009b) were re-analyzed using a MCMC analytical approach similar to the one performed in Yang et al. (2012). The key differences between the new analysis and the original Yang et al. (2012) analysis were: (1) the incorporation of an additional parameter in the analysis of the *in*

vitro metabolism data (Kg) to describe the rate of transfer of chloroprene from the headspace to the media in the metabolism studies, (2) the use of updated tissue microsomal protein concentrations for scaling the *in vitro* results to *in vivo* values appropriate for the PBPK model, and (3) the adoption of a previously published approach for estimating the metabolism parameters in the human lung (Andersen et al. 1987).

Re-estimation of in vitro metabolism parameters: Schlosser et al. (1993) suggested that mass transport limitations should be assessed when estimating metabolism from *in vitro* experiments conducted with volatile compounds where there is an air:liquid interface. Since mass transport limitation was not addressed in the *in vitro* metabolism studies conducted with chloroprene (Himmelstein et al. 2004a, Yang et al. (2012), a new experimental study was performed to estimate a Kg for chloroprene following a protocol similar to that in Schlosser et al. (1993). The new experimental study, which is described in Supplemental Materials C and D, resulted in an estimated value of 0.024 L/hr for Kg, similar to the value previously reported for benzene (Schlosser et al. 1993). However, the experimental value of Kg was not consistent with the high rates of liver metabolism observed at low concentrations of chloroprene; that is, the mass transport associated with a Kg of 0.024 L/hr was too slow to support the observed rates of metabolism in the media. It is possible that the presence of microsomes in the media in the metabolism studies may have increased the rate of uptake of chloroprene through lipophilic binding.

As an alternative approach for determining the Kg in the original metabolism studies, a re-analysis of the *in vitro* metabolism data was performed to simultaneously estimate Kg along with the metabolism parameters, Vmax and Km. However, a high degree of collinearity between Km and Kg made it impossible to estimate all three parameters independently. To resolve this interdependency, a re-analysis of the data on metabolism in the male mouse liver (the strongest data set for parameter estimation) was performed to estimate Vmax and Kg, with Km set to a fixed value of 1.0 μmol/L. The value of Km was based on a literature review of compounds with structural similarities to chloroprene, specifically, halogenated alkanes and alkenes, which found that the values of Km estimated for these compounds from a variety of *in vivo* studies in mice, rats and humans ranged from 1 to 5 μmol/L (Andersen et al. 1987b, 1991, 1994; Clewell et al.

2001; Corley et al. 1990; David et al. 2006; D'Souza et al. 1987, 1988; D'Souza and Andersen 1988; Gargas et al. 1986, 1990; Gargas and Andersen 1989; Lilly et al. 1997, 1998; Marino et al. 2006). The strongest data were from studies of mutual metabolic inhibition in co-exposures to trichloroethylene and dichloroethylene (Andersen et al. 1987b), which estimated Kms of 1.9 and 1.0 μmol/L, respectively. Consistent with these estimates, a Km of 1.6 μmol/L was used by the USEPA in their risk assessment for vinyl chloride (USEPA 2000). The resulting value of Kg (0.45 L/hr) from this analysis (Supplemental Materials C) was then used in the re-estimation of the metabolism parameters for all tissues. The results of the new *in vitro* metabolism parameter estimation are provided in Table S-3 in Supplemental Materials B.

Selection of tissue scaling parameters: Based on a review of the literature (Supplemental Materials E), an updated set of scaling parameters was chosen: 35, 45, and 40 mg protein/g liver for mice, rats, and humans, respectively, 20 mg protein/g lung for all species, and 11 mg protein/g kidney for all species (Medinsky et al. 1994, Houston and Galetin 2008, Barter et al. 2008, Yoon et al. 2007, Scotcher et al. 2017). The in vivo metabolism parameters derived using the revised scaling parameters are listed in Table S-4 in Supplemental Materials B and the IVIVE calculations are provided in Supplemental Materials F.

Estimation of chloroprene metabolism in the human lung: Unfortunately, we found that the extremely low rates of chloroprene metabolism observed in the human lung (Himmelstein et al. 2004a) made parameter estimation for this tissue highly uncertain. The 95% confidence interval for human lung metabolism in the new MCMC analysis ranged from near zero (7.5E-23) to 0.44 L/hr/g microsomal protein, with a mean that was also near zero (1.5E-11). Therefore, we estimated the metabolism parameter for the human lung using the approach used in the USEPA (2011) risk assessment for methylene chloride, which relied on the PBPK model developed by Andersen et al. (1987), In that model, the Km for metabolism in the human lung was assumed to be the same as the Km in the human liver, and the Vmax in the human lung was calculated from the Vmax in the human liver using a parameter (A1) derived from the ratio of the specific activities for metabolism of 7-ethoxycoumarin, a well-studied CYP2E1 substrate, in liver and lung (Lorenz et al. 1984).

Model Simulations

The previously published version of the chloroprene PBPK model (Yang et al. 2012), which was written in the Advanced Continuous Simulation Language (ACSL), was translated into R, an open source programming language, to improve its portability. The R code for the model is included in Supplemental Materials G.

To model the experimental data from the nose-only inhalation exposures reported here, only the alveolar ventilation and cardiac output were altered. The average ventilation rate measured in the mice during the study was used to calculate an alveolar ventilation for use in the model, assuming 2/3 of total ventilation is alveolar (Brown et al. 1997), and the cardiac output was then calculated by dividing the alveolar ventilation by the V/Q ratio from Marino et al. (2006), as described in the results.

Parameter sensitivity analysis was conducted with the model under two scenarios: (1) the prediction of blood concentrations in the mouse nose-only study, and (2) the prediction of dose metrics for the mouse bioassay exposures and for the human at 1 ppm continuous exposure. The results are displayed as normalized sensitivity coefficients (fractional change in prediction divided by fractional change in parameter) for parameters with a coefficient greater than 0.1 in absolute magnitude. A positive coefficient indicates the direction of change of the prediction is the same as the direction of change of the parameter. The parameters were changed by 1%, one at a time.

Risk Assessment Application

Consistent with previous PBPK modeling of chloroprene (Himmelstein et al. 2004b, Yang et al 2012), the dose metric calculated with the PBPK model for derivation of an IUR is micromoles of chloroprene metabolized in the lung per gram lung per day. This dose metric was chosen because the lung is the tissue with the highest tumor incidence in the chloroprene inhalation bioassays (NTP 1998) and the carcinogenicity of chloroprene in rodents is believed to result from its metabolism to reactive epoxides in the target tissue (Himmelstein et al. 2004a,b). The dose metric selected for chloroprene is consistent with the dose metrics used in previous PBPK-based risk assessments for both vinyl chloride (Clewell et al. 2001, USEPA 2000) and methylene chloride (Andersen et al. 1987, USEPA 2011), which were also based on the production of reactive metabolites.

To estimate an IUR, the PBPK model was first used to simulate the NTP (1998) bioassay exposures (12.8, 32 and 80 ppm; 6 hours/day, 5 days/week) and calculate the corresponding target tissue dose metrics (in this case, average daily production of epoxide metabolites in the lung per gram lung). Consistent with USEPA practice, the PBPK-based target tissue dose metrics were then used in place of the air concentrations in BMDS, the USEPA's Benchmark Dose modeling program, to estimate a 95% lower-bound estimate of the dose metric associated with a tumor risk of 0.01 (the $BMDL_{01}$). The PBPK model was then used to estimate the same target tissue dose metric in a human exposed continuously to chloroprene at a concentration of 1 $\mu g/m^3$ for their lifetime. The IUR was then estimated by the following formula:

Risk at 1 ug/m^3 = 0.01 * (human dose metric at 1 ug/m^3) / (mouse dose metric at $BMDL_{01}$)

Results

Chloroprene Exposure Atmospheres

Chloroprene concentrations were monitored in the nose only chambers during the 13, 32, and 90 ppm exposures, as well as in the control nose-only tower. All three target concentrations were well within 10% of their nominal levels.

Plethysmography

Figure 2 shows the measured minute volumes for the three exposure groups and controls. The data is represented as average values (diamonds) with standard deviation error bars. The data is provided in Table S-5 in Supplemental Materials B. There is no evidence of a concentration-related effect of short-term exposure to chloroprene on ventilation in mice. The average ventilation rate across all four exposure groups, including controls, was 56.2 mL/min. The average body weight for the mice in the study was 22g; therefore, this ventilation rate equates to a model parameter for alveolar ventilation (QPC) of 39.4 $L/hr/bw^{3/4}$. The corresponding model value of QCC in this study is obtained by dividing QPC by the V/Q ratio of 1.45 for the mouse (Marino et al. 2006), yielding a value for QCC of 27.2 $L/hr/bw^{3/4}$, which compares well with the QCC of 24.2 estimated for mouse exposures to methylene chloride (Marino et al. 2006).

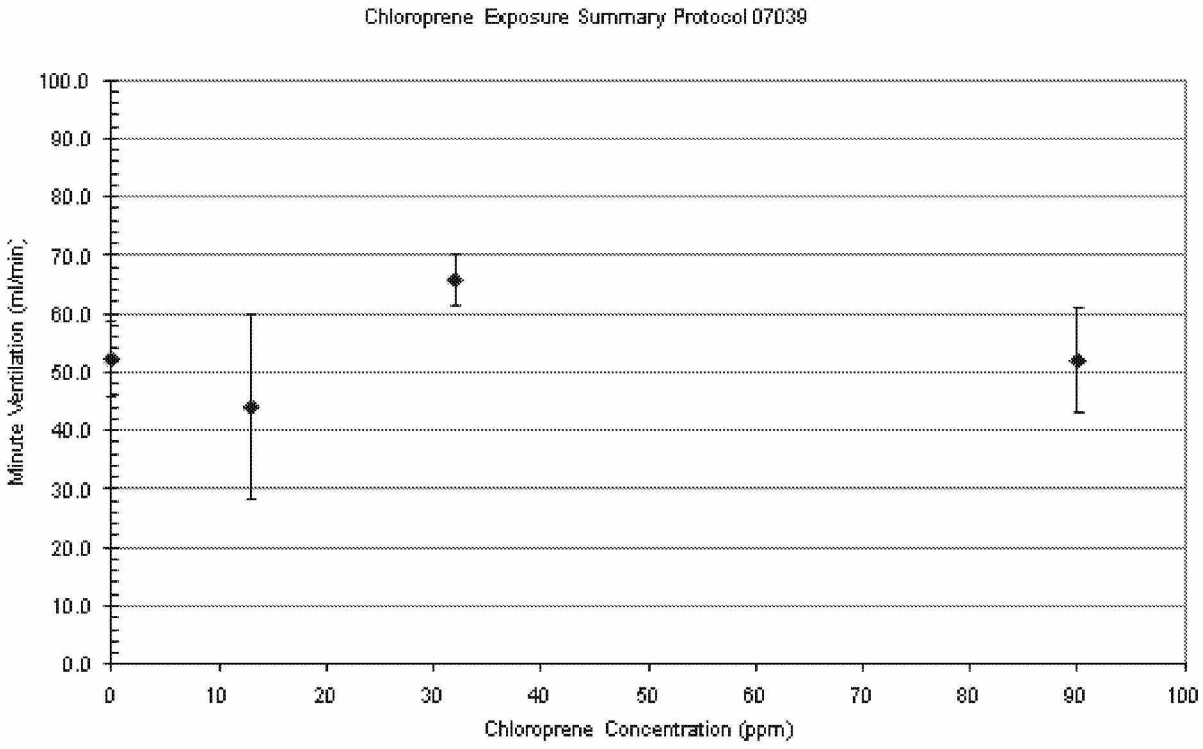

Figure 2: Measured minute ventilation during exposures.

Arterial Blood Chloroprene Concentrations

Figure 3 shows the average CD blood concentrations for all three single day exposures (Data are provided in Table S-6 of Supplemental Materials B). Average blood chloroprene concentrations are represented by the symbols with standard deviations for each treatment group represented with error bars.

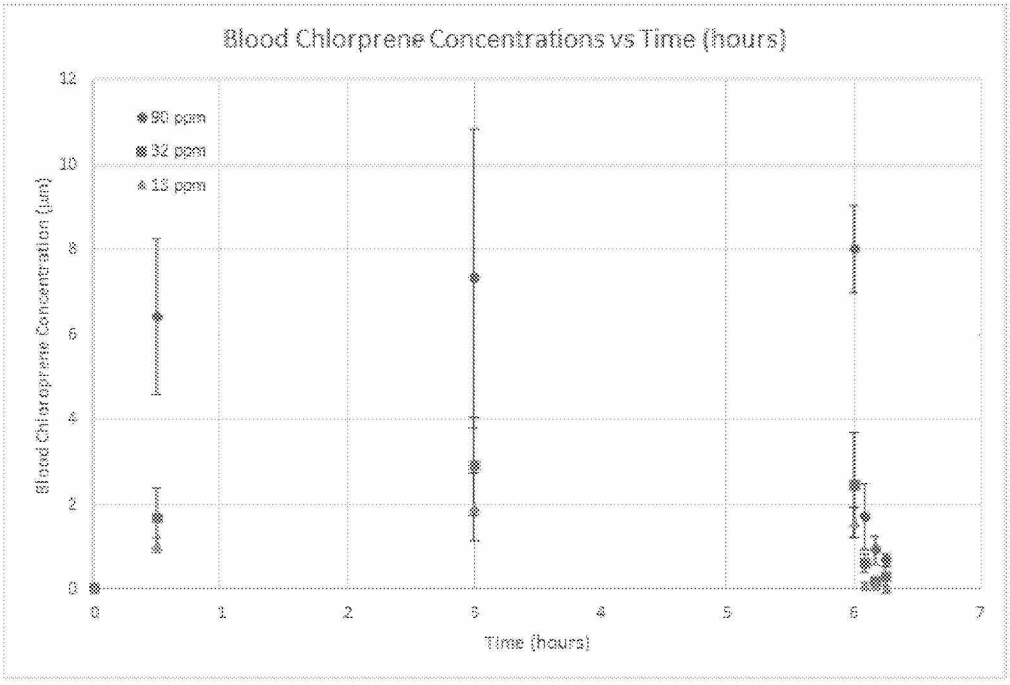

Figure 3. Arterial blood chloroprene concentrations during and following a single nose-only exposure of female B6C3F1 mice to chloroprene at 13, 32 and 90 ppm for 6 hours. Average blood chloroprene concentrations (symbols) and standard deviations (error bars) are shown for each treatment group.

PBPK Modeling of the Nose-Only Inhalation Study

The nose-only study described above was simulated with the chloroprene PBPK model using the parameters in Tables S1, S2, and S4, except for QPC and QCC, where the study-specific values derived from the plethysmography data were used. As shown in Figure 4, using only *in vitro*-derived metabolism and partitioning parameters the model predictions for arterial blood concentrations during and after the 6-hr chloroprene exposures are in good agreement with the data collected in the study, with model predictions generally lying within roughly a factor of two of the means of the experimental data. It was not necessary to adjust any of the model parameters to provide agreement with the new data.

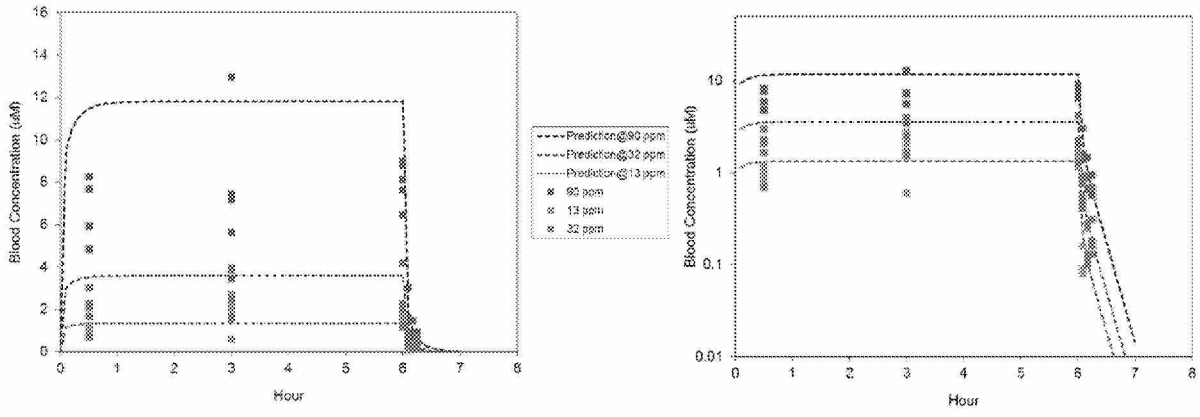

Figure 4. PBPK model predicted (dotted lines) and measured (symbols) arterial blood concentrations during and following 6-hr exposures of B6C3F1 mice to chloroprene at 13, 32 and 90 ppm. The same data and model predictions are shown using a linear y axis (left) and a logarithmic y axis (right). The linear plot provides a better comparison for concentrations, whereas the logarithmic plot provides a clearer comparison for the post-exposure clearance.

PBPK Model Parameter Sensitivity

As shown in Figure 5, when simulating the nose-only exposures only 4 model parameters have sensitivity coefficients greater than 0.1 in absolute magnitude: alveolar ventilation, cardiac output, blood:air partition coefficient and fractional blood flow to liver. All of these parameters were either directly measured or based on data from the literature, as described in the Methods, and can be considered to have low uncertainty. When predicting lung dose metrics in the female mouse (Figure 6), the sensitive parameters include the same parameters as those for the predictions of blood concentrations, with the addition of the parameters for lung metabolism and the body weight. The sensitive parameters for predictions of lung dose metrics in the human (Figure 7) are the same as those in the mouse, except that a single clearance parameter is used in the human due to the low rate of metabolism in the human lung. These analyses of the sensitivity of the model to uncertainty in its parameters suggest that performing a human *in vivo* validation study would be unlikely to provide a significant added value for model evaluation.

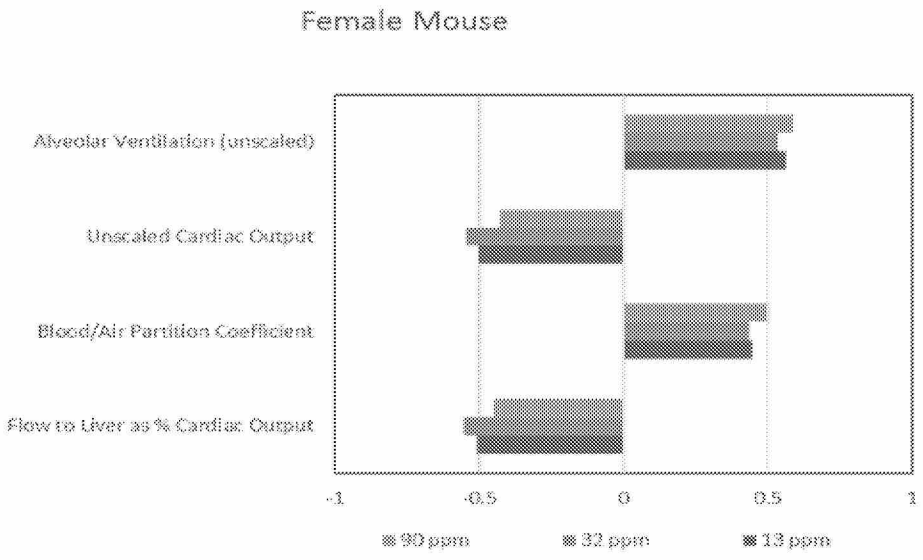

Figure 5. Parameter sensitivity coefficients for the chloroprene PBPK model for the prediction of arterial blood concentrations in the nose-only study.

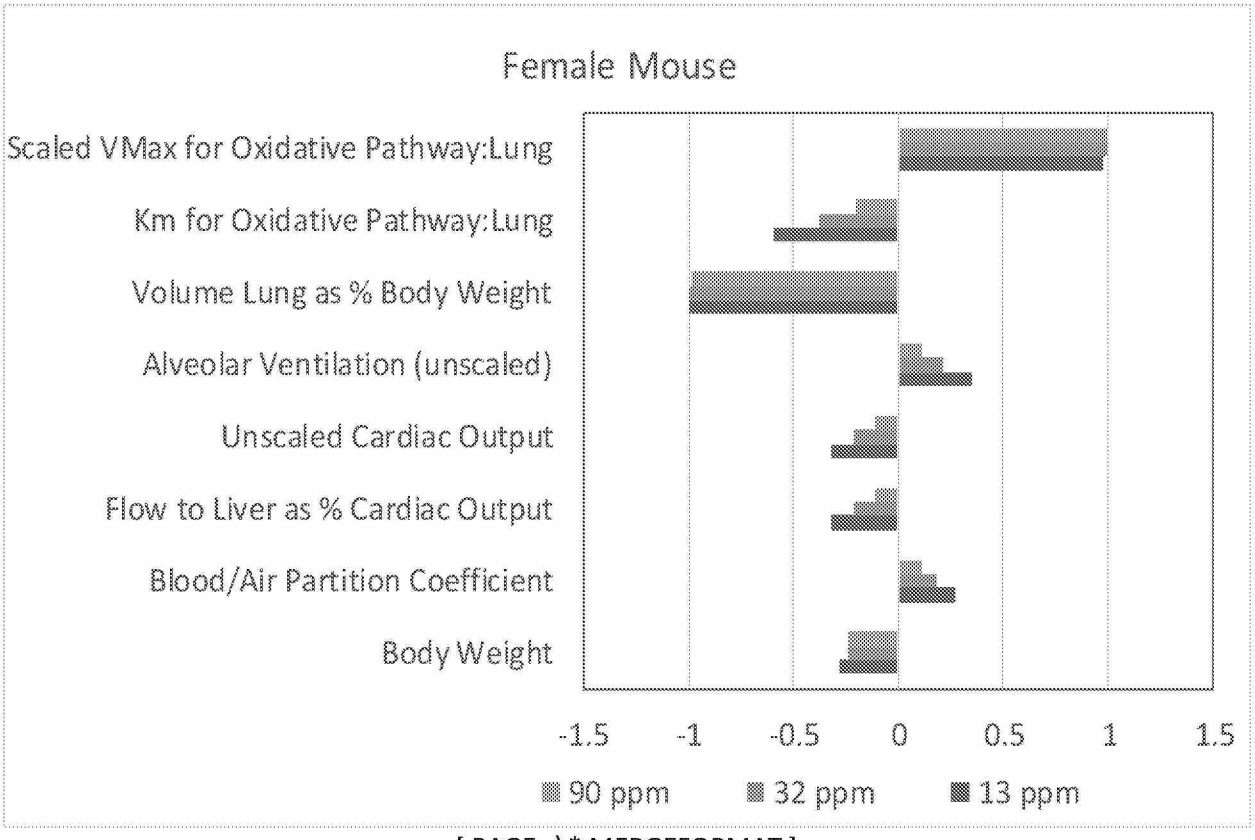

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Figure 6. Parameter sensitivity coefficients for the chloroprene PBPK model for the prediction of lung dose metrics in the female mouse for exposures in the 2-year bioassay.

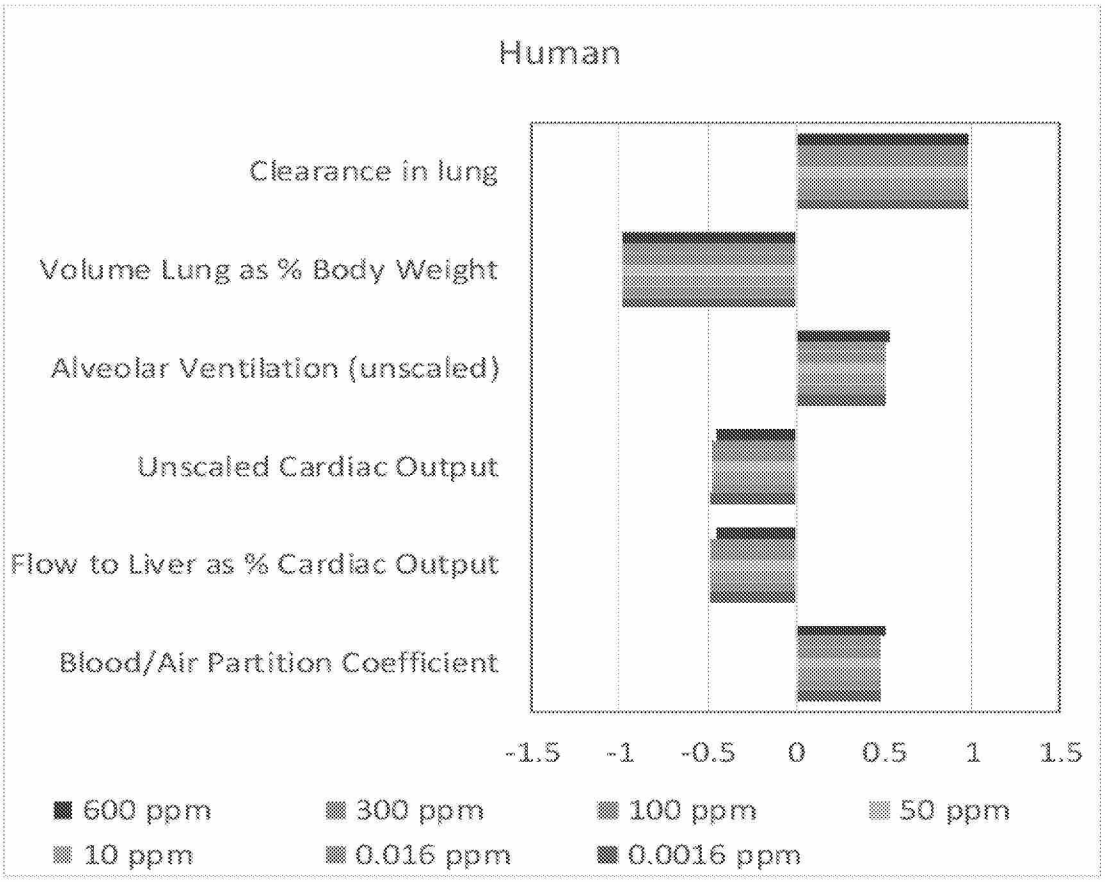

Figure 7. Parameter sensitivity coefficients for the chloroprene PBPK model for the prediction of lung dose metrics in the human for continuous exposure at 1 ppm.

PBPK-Based Risk Assessment for Chloroprene Lung Carcinogenicity

The dose metrics for lung metabolism in the female mouse bioassay and for human continuous exposure are shown in Table 1. These estimates were obtained with the chloroprene PBPK model using the parameters in Tables S1, S2, and S4.

Exposure	Concentration	Dose metric
Female mouse bioassay	12.3 ppm	1.20
	32 ppm	1.91
	80 ppm	2.52
$BMDL_{01}$		**0.0114**
Human continuous	100 ppm	0.041
	10 ppm	0.031
	1 ppm	0.0091
	0.1 ppm	$1.13x10^{-3}$
	0.01 ppm	$1.15x10^{-4}$
	1 ppb	$1.16x10^{-5}$
	1 $\mu g/m^3$	$3.20x10^{-6}$
IUR $(\mu g/m^3)^{-1}$		**$2.81x10^{-6}$**

Table 1. Dose metrics for lung metabolism (average mg metabolized per gram lung per day) in the female mouse bioassay and for human continuous exposures. Also shown are the $BMDL_{01}$ calculated from the mouse dose metrics and the resulting IUR.

Using the dose metrics from the model, the $BMDL_{01}$ in the mouse estimated with BMDS was 0.0114, resulting in an estimated IUR of $0.01*3.20x10^{-6}/0.0114 = 2.81\ x10^{-6}$, a factor of 178 lower than the USEPA (2010) IUR of 5.0E-4.

Discussion

In this study we characterized the time course blood concentrations of chloroprene in female B6C3F1 mice during and following a single 6-hour nose-only inhalation exposure over the range of concentrations used in the NTP (1998) bioassays. These data, including both arterial whole blood concentrations and respiratory parameters (breathing frequency and tidal volume) during and after these exposures provide a reliable basis for evaluating the ability of the chloroprene PBPK model to predict *in vivo* pharmacokinetics in the bioassays. We have then applied the PBPK model in an inhalation cancer risk assessment that considers species differences in pharmacokinetics. The IUR obtained with the PBPK model was 178-fold lower than the IUR published by EPA (2010) based on inhaled chloroprene concentration. The principal reason for the lower human risks estimated with the PBPK model as compared to the EPA (2010) assessment, which was based on inhaled chloroprene concentration, is the use of a pharmacokinetic dose metric for cross-species extrapolation that considers the impact of metabolic differences. The use of a PBPK model for this purpose is consistent with the conclusion of the National Academy of Science (NRC 1987) that: "relevant PBPK data can be used to reduce uncertainty in extrapolation and risk assessment."

It is important to note that, due to the low rates of metabolism in the *in vitro* assays for the rat and human lung, it is only possible to estimate a pseudo-first-order clearance for these tissues. Therefore, the original chloroprene model (Himmelstein et al. 2004b, Yang et al. 2012) used a linear description of metabolism in these tissues, which is only appropriate in the concentration range below the Km in the lung, a parameter that is highly uncertain in the rat and human. Thus model-based metabolism predictions for human exposures significantly greater than 1 ppm would greatly overestimate the associated risk. One approach for dealing with the inability to estimate the value of Km in the human lung is to use the value of Km estimated in the human

liver. This approach was used in the PBPK model for methylene chloride (Andersen et al. 1987) and in the present analysis. The impact of saturable metabolism on human dose metric predictions is shown in Figure 8. Without estimating a value for Km, the model-predicted risks above 1 ppm would continue to increase at a biologically implausible rate.

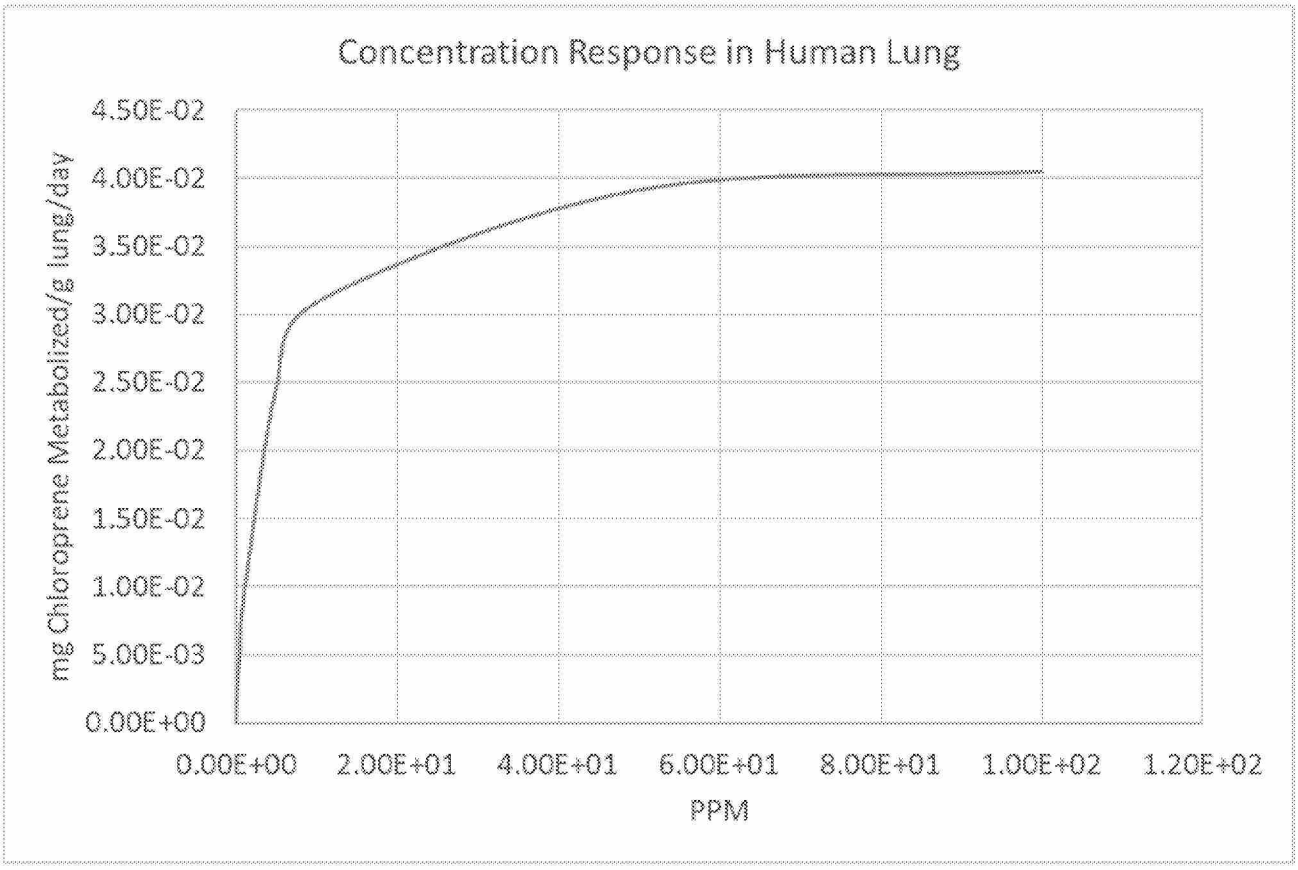

Figure 8. Inhaled concentration dependence of lung metabolism in the human for continuous exposures to chloroprene predicted with the PBPK model.

Interestingly, comparison of the Kms for chloroprene in liver and lung for male and female mice (Table S-3), which are based on the strongest data sets for estimating Kms, suggests that Km may be higher (lower affinity) in the mouse lung than in the mouse liver. This difference in apparent affinities in mouse liver and lung is consistent with differences in the relative tissue abundances of the murine CYP2E1 and CYP2F isozymes, both of which exhibit high affinities for chlorinated alkenes (Yoon et al. 2007). Whereas CYP2E1 is the predominant high affinity

isozyme in the mouse liver, CYP2F is the predominant high affinity isozyme in the mouse lung (Yoon et al. 2007) and, consistent with the estimated Kms for chloroprene, the affinity of rCYP2E1 is roughly 3-fold higher (lower Km) than rCYP2F2 (Simmonds et al. 2004). Howeve4r, since there is no evidence of CYP2F activity in the primate lung (Baldwin et al. 2004), no difference in Kms in the human lung and liver would be expected, so the estimation of human lung Km based on the human liver Km is appropriate.

Not unexpectedly, in our re-analysis we found that the extremely low rates of chloroprene metabolism observed *in vitro* in the human lung (Himmelstein et al. 2004a) made parameter estimation for this tissue highly uncertain. The 95% confidence interval for human lung metabolism in the new MCMC analysis ranges from near zero (7.5E-23) to 0.44 L/hr/g microsomal protein, with a mean that is also near zero (1.5E-11). Therefore, we estimated the metabolism parameter for the human lung using the same approach as the USEPA (2011) risk assessment for methylene chloride; that is, the Km for metabolism in the human lung was assumed to be the same as the Km in the human liver, and the Vmax in the human lung was calculated from the Vmax in the human liver using a parameter (A1) derived from the ratio of the specific activities for metabolism of 7-ethoxycoumarin, a well-studied CYP2E1 substrate, in liver and lung (Lorenz et al. 1984). Using the human value of A1 (0.00143), together with the estimated values of Vmax and Km in the human liver from the MCMC analysis (0.053 µmol/hr/mg protein and 0.38 µmol/L), results in a metabolic clearance in the lung of 0.20 L/hr/g microsomal protein. This human lung metabolism estimate is similar to the value of 0.32 L/hr/g microsomal protein previously estimated for chloroprene by Yang et al. (2012) and is within the confidence interval estimated by our new analysis of the *in vitro* data. In support of the applicability of A1 to chloroprene, the value of A1 in the male mouse (0.414) from Lorenz et al. (1984) is close to the ratio of the *in vitro* Vmax in the lung and liver of the male mouse in our new analysis (0.56, see Table S-3).

Selection of Dose Metric

The dose metric calculated with the PBPK model in this analysis is micromoles of chloroprene metabolized in the lung per gram lung per day (Himmelstein et al. 2004b, Yang et al 2012). This dose metric was chosen because (1) the lung is the tissue with the highest tumor incidence in the

chloroprene inhalation bioassays (NTP 1998) and (2) the carcinogenicity of chloroprene in rodents is believed to result from its metabolism to reactive epoxides in the target tissue (Himmelstein et al. 2004a,b). The dose metric selected for chloroprene is consistent with the dose metrics used in previous PBPK-based risk assessments for both vinyl chloride (Clewell et al. 2001, USEPA 2000) and methylene chloride (Andersen et al. 1987, USEPA 2011), which were also based on the rate of production of reactive metabolites. The dose metric selected for the liver carcinogenicity of vinyl chloride was total mg vinyl chloride metabolized per kg liver per day, representing the production of the reactive chloroethylene epoxide. Due to the presence of chlorine in the epoxides generated from the metabolism of chloroprene, they are considered likely to have a reactivity comparable to vinyl chloride (Haley 1978, Plugge and Jaeger 1979). The methylene chloride dose metric was average daily metabolism by the glutathione conjugation pathway in the lung per gram lung, which was selected based on evidence that the carcinogenicity of methylene chloride was associated with the local production of a reactive metabolite from the glutathione conjugate of methylene chloride. As with vinyl chloride and chloroprene, the assumption inherent in the dose metric was that the reactive metabolite would be completely consumed within the tissue where it was generated.

Himmelstein et al. (2004b) have previously demonstrated that using the PBPK dose metric is able to harmonize the dose-responses for lung tumors in mice, rats and hamsters. However, they only had metabolism data for male animals. Figure 8 shows an update of the analysis from Himmelstein et al. (2004b) that includes the results for the female mouse and rat. While the revised PBPK model is still able to demonstrate the consistency of the tumor incidence across male animals of different species and strains, female mice exhibit a higher tumor incidence than male mice at the same rate of lung metabolism.

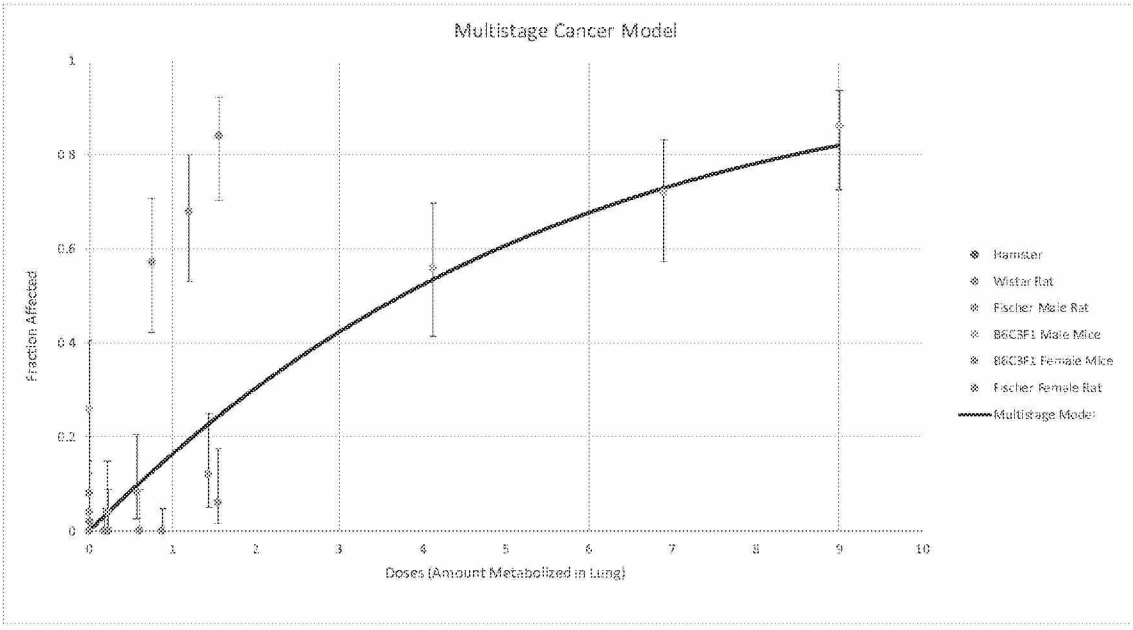

Figure 8. Comparison of Dose-Response for Lung Tumors in Chloroprene Bioassays in Rodents.

This discrepancy could indicate either of two possibilities: (1) the selected dose metric, rate of metabolism of chloroprene in the lung, is incorrect, or (2) the female mouse lung is more sensitive to the effects of chloroprene metabolites than the male mouse lung. Relatively few studies have been conducted to explore gender differences in the responses to chemical insult in the mouse lung. However, Yamada et al. 2017, provides evidence of a proliferative response of Club cells to the toxicity of permethrin in the female mouse lung that is not observed in the male mouse lung, and studies of naphthalene lung toxicity have demonstrated a greater sensitivity of the female mouse lung to both acute and repeated toxicity (Van Winkle et al. 2002, Sutherland et al. 2012). The greater susceptibility to a proliferative response to lung toxicity in the female mouse appears to result from gender differences in the tissue response to damage rather than metabolism (Laura Van Winkle, personal communication). A study of the genomic responses in the lungs of female mice and rats to inhaled chloroprene (Thomas et al. 2013) also demonstrated a greater pharmacodynamic sensitivity of the female mouse. In this study, female mice and rats were exposed for up to three weeks to inhaled chloroprene concentrations that were chosen to result in similar rates of epoxide production in the two species. The study found that while the most sensitive tissue responses occurred at similar values of the metabolism dose metric,

transcriptional evidence of oxidative stress occurred at much lower concentrations in the female mouse. The more sensitive response of the female mouse to oxidative stress and to a proliferative response may underlie the apparent potency difference indicated by Figure 8. Using the metabolism dose metric effectively ignores the greater sensitivity of the female mouse, which is health protective, since the greater sensitivity of the female mouse results in a lower BMDL01 than would be obtained from the male mouse.

The risk assessment for vinyl chloride (USEPA 2000) demonstrated that the use of a PBPK model to estimate target tissue dose (based on total metabolism per gram liver per day) was able to produce similar human risk estimates using data from animal bioassays and human occupational exposures. As a similar test of the chloroprene PBPK model to support cross-species extrapolation, Allen et al. (2014) used a statistical maximum likelihood approach to compare risk estimates obtained using external (air concentration) and internal (PBPK model estimated) metrics for the female mouse bioassay and human occupational exposures. The analysis concluded that if inhaled concentration was used as the dose metric, the estimates of human cancer risk using animal and human data were statistically significantly different, whereas using the PBPK metric consistent risk estimates were obtained across species. As with vinyl chloride, the use of the PBPK-based metric effectively reconciled the differences in mouse and human low-dose risk estimates.

Use of *In Vitro* Metabolism Data

The most notable aspect of the chloroprene PBPK model is that, apart from the physiological parameters, the parameters in the model are based on data derived solely from *in vitro* studies. The PBPK model for chloroprene is structurally similar to the PBPK model for methylene chloride (Andersen et al. 1987) and, just as in the case of the methylene chloride risk assessment, model predictions needed to support a risk assessment are critically dependent on parameters that can only be derived from *in vitro* metabolism experiments.

In their analysis of *in vitro* data on chloroprene metabolism, Yang et al. (2012) employed both a standard frequentist approach (referred to in their analysis as a "deterministic" approach) and an approach that used a Markov Chain Monte Carlo (MCMC) method (referred to as a

"probabilistic" approach) with non-informative prior distributions for all estimated parameters. The use of non-informative priors allows this Bayesian approach to be interpreted from a frequentist perspective. As stated in the Yang et al. document, the two methods were compared to demonstrate that the they provided consistent estimates of metabolic parameter values. Yang et al. (2012) then relied on the MCMC-based estimates for developing dose metrics for chloroprene exposures in mouse, rat and human. Because it seeks a global optimum using a probabilistic direct search algorithm, MCMC is less likely than deterministic search algorithms to converge on a local optimum. Moreover, when used with non-informative priors, as in Yang et al. (2012), the posterior distribution represents the likelihood distribution for the parameter, and the mode of the distribution represents the maximum likelihood estimate (MLE). As pointed out in Chiu et al. (2007), the Bayesian approach, in principle, yields a more global characterization of parameter uncertainty than the local, linearized variance estimates provided by traditional optimization routines, which should be viewed as lower bound estimates of true parameter uncertainty. Because of its superior properties, we have also relied on the MCMC approach in our re-analysis of the original *in vitro* metabolism data. The key difference between the MCMC analysis performed in this study and the original analysis (Yang et al. 2012) was that this re-analysis included an additional parameter (Kg) for the *in vitro* experiments, representing the potential for a mass transport limitation for uptake of chloroprene from the air in the metabolism vials.

Use of *In Vivo* Data for PBPK Model Validation

PBPK modeling has now been applied in risk assessments for a variety of environmental chemicals by regulatory agencies worldwide. The development of these models has typically required the use of *in vivo* experimental animal and/or human data to estimate key kinetic parameters such as uptake, metabolism and elimination. Some agencies also require the use of separate *in vivo* data to demonstrate model validity. However, it has become increasingly difficult to conduct controlled exposures of human subjects to chemicals of concern, other than for pharmaceuticals. The need for live animal studies is also being challenged, particularly in the EU, due to both ethical and practical (cost, throughput) concerns. Therefore, requirements for *in vivo* testing will increasingly limit the potential application of PBPK modeling in risk

assessment, and agencies will need to consider whether *in vivo* validation data is truly necessary for assessing the fitness of a model for the specific purpose of its use in a particular risk assessment.

The original chloroprene PBPK model (Himmelstein et al. 2004b) was not used by USEPA (2010) because the agency considered it necessary to have blood or tissue time course concentration data from an *in vivo* study to adequately validate the model. The study reported here was conducted to address this requirement and we have now demonstrated that the chloroprene PBPK model accurately simulates these *in vivo* blood time course validation data.

No *in vivo* validation data for chloroprene is available in the human, and it is unlikely that such a study could be performed given the current classification of chloroprene as "likely to be a carcinogen" (USEPA 2010). However, the sensitivity analyses reported here suggest that such a study would not provide significant added value for demonstrating that the PBPK model is fit for purpose for a chloroprene risk assessment. The validity of the model instead derives from the biological validity of the physiological and biochemical underpinnings of the model structure and parameters. The key parameters for performing a risk assessment for chloroprene are those for lung metabolism, and a human *in vivo* study would not be able to provide informative data for those parameters. As shown in Figure 5, blood concentrations of chloroprene associated with inhalation are insensitive to lung metabolism, and depend only on alveolar ventilation, cardiac output, blood:air partition coefficient and fractional blood flow to liver.

The limited value of human *in vivo* data for determining whether a PBPK model is fit for purpose in a risk assessment based on target tissue metabolism was also an issue during the development of the PBPK model of methylene chloride (Andersen et al. 1987), where a similar dose metric was used: average daily metabolism of methylene chloride by glutathione transferase (GST) in the lung per gram lung. Although the model accurately reproduced blood and exhaled air concentration time-course data from multiple studies with human subjects, the *in vivo* data were not adequate to estimate the rates of GST metabolism in the liver and lung. Instead, it was necessary to estimate the rate of GST metabolism in the human liver by allometric scaling from animal data (Andersen et al. 1987), and to then estimate the rate of GST metabolism in the

human lung using the ratio of specific activities for GST metabolism in liver and lung measured *in vitro* by Lorenz et al. (1984).

At the time the methylene chloride PBPK model was developed, the use of *in vitro* data to predict *in vivo* metabolism was a relatively new concept, but in the intervening years it has become common practice both for pharmaceuticals (Rostami-Hodjegan 2012) and environmental chemicals (Yoon et al. 2012). Nevertheless, regulatory agency acceptance of PBPK models that are not based primarily on *in vivo* data still presents a challenge. Going forward it will be important to develop a consensus on standard practices for IVIVE of metabolism in PBPK modeling in order to assist agencies in their evaluations. Additionally, PBPK model evaluations should make greater use of uncertainty analyses to estimate the potential reduction in model uncertainty associated with the collection of additional data; that is, to determine the added value of a proposed study.

Conclusion

A PBPK model of chloroprene that relies solely on data from *in vitro* studies for its metabolism parameters accurately predicts the *in vivo* time course for chloroprene in the blood of female mice exposed by nose-only inhalation to the 3 concentrations used in the chloroprene 2-year cancer bioassay. The human lung cancer risk estimated using the PBPK model is lower than the USEPA (2010) risk estimates based on inhaled concentration by a factor of 178. Similar large differences between PBPK-based risk estimates and estimates based on inhaled concentration have been seen in previous inhalation risk assessments for chemicals where toxicity results from the production of reactive metabolites (Andersen et al. 1987, Clewell et al. 2001). Given the potentially high impact of species differences in pharmacokinetics on estimates of human risk and the potentially limited value of *in vivo* data, particularly human data, for validating some PBPK models, future requirements for validation of a PBPK model using *in vivo* data should be evaluated on a case-by-case basis to determine the potential added value of the studies before making them a condition for acceptance of a PBPK model in a risk assessment.

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Supplemental Materials

A. Nose-only Inhalation Study Report (IISRP 2009a)

B. Supplemental Tables

C. Re-estimation of Metabolism Parameters

D. Kg Study Report

E. IVIVE Literature Review

F. Metabolism Parameter Calculations

G. PBPK Model Code

A. Supplemental Tables

Table S-1: Physiological Parameters for PBPK Model

Parameter	Abbreviation	Units	Mice		Source	Rats		Source	Humans	Source
			Male	Female		Male	Female			
Body Weight	BW	kg	0.03	0.03	Brown et al. 1997 (Table 1)	0.25	0.25	Brown et al. 1997 (p.413)	70	Brown et al. 1997 (p.415)
Alveolar Ventalation	QPC	$L/h/kg^{0.75}$	29.1	29.1	Brown et al. 1997 (Table 31)	22.4	22.4	Brown et al. 1997 (Table 31)	24.0	Clewell et al. 2001 (Table 1)
Cardiac Output	QCC	$L/h/kg^{0.75}$	20.1	20.1	Marino et al. 2006 (QPC/QCC = 1.45)	18.7	18.7	Brown et al. 1997 (Table 22)	16.5	Clewell et al. 2001 (Table 1)
FRACTIONAL BLOOD FLOWS TO TISSUES										
Flow to Liver as fraction Cardiac Output	QLC	unitless	0.161	0.161	Brown et al. 1997 (Table 23)	0.183	0.183	Brown et al. 1997 (Table 23)	0.227	Brown et al. 1997 (Table 23)
Flow to Fat as fraction Cardiac Output	QFC	unitless	0.07	0.07	Brown et al. 1997 (Table 23; Same as rat value)	0.07	0.07	Brown et al. 1997 (Table 23)	0.052	Brown et al. 1997 (Table 23)
Flow to Slow as fraction Cardiac Output	QSC	unitless	0.159	0.159	Brown et al. 1997 (Table 23); Same as that reported for muscle	0.278	0.278	Brown et al. 1997 (Table 23); Same as that reported for muscle	0.191	Brown et al. 1997 (Table 23); Same as that reported for muscle
Flow to Kidney as fraction Cardiac Output	QKC	unitless	0.09	0.09	Brown et al. 1997 (Table 23)	0.14	0.14	Brown et al. 1997 (Table 23)	0.175	Brown et al. 1997 (Table 23)

FRACTIONAL VOLUMES OF TISSUES										
Volume Liver as fraction Body Weight	VLC	unitless	0.055	0.055	Brown et al. 1997 (Table 4)	0.0366	0.0366	Brown et al. 1997 (Table 5)	0.0257	Brown et al. 1997 (Table 7)
Volume Lung as fraction Body Weight	VLUC	unitless	0.0073	0.0073	Brown et al. 1997 (Table 4)	0.005	0.005	Brown et al. 1997 (Table 5)	0.0076	Brown et al. 1997 (Table 7)
Volume Fat as fraction Body Weight	VFC	unitless	0.1	0.1	Brown et al. 1997 (Table 10)	0.1	0.1	Brown et al. 1997 (Table 13)	0.27	Brown et al. 1997 (Table14); Average of total male and female
Volume Rapid Perfused as fraction Body Weight	VRC	unitless	0.08098	0.08098	Brown et al. 1997 (Table 4); Sum of adrenals, brain, stomach, small intestine, large intestine, heart, lungs, pancreas, spleen and thyroid	0.04644	0.04644	Brown et al. 1997 (Table 5); Sum of adrenals, brain, stomach, small intestine, large intestine, heart, lungs, pancreas, spleen and thyroid	0.0533	Brown et al. 1997 (Table 7); Sum of adrenals, brain, stomach, small intestine, large intestine, heart, lungs, pancreas, spleen and thyroid
Volume Slow Perfused as fraction Body Weight	VSC	unitless	0.384	0.384	Brown et al. 1997 (Table 4); Same as that reported for muscle	0.4	0.4	Brown et al. 1997 (Table 5); Same as that reported for muscle	0.4	Brown et al. 1997 (Table 7); Same as that reported for muscle
Volume Kidney as fraction Body Weight	VKC	unitless	0.0167	0.0167	Brown et al. 1997 (Table 4)	0.0073	0.0073	Brown et al. 1997 (Table 5)	0.0044	Brown et al. 1997 (Table 7)

Table S-2: Partition Coefficients for PBPK Model

	Mice	Rats	Humans
Blood:Air	7.83	7.35	4.54
Lung:Blood	2.38	1.85	2.94
Liver:Blood	1.26	1.58	2.37
Fat:Blood	17.35	16.99	28.65
Muscle:Blood [a]	0.59	0.60	1.00
Kidney:Blood [b]	1.76	2.29	2.67

[a] used for slowly perfused tissues; [b] used for rapidly perfused tissues

Table S-3: In Vitro Metabolism Parameters

Sex & Species	Parameter	Source	
		Yang et al. 2012 MCMC Mean	This Analysis Mean (95% Confidence Interval)
Female mouse	Vmax, liver (µmol/h/mg)	0.13	0.11 (0.093 - 0.14)
	Vmax, lung (µmol/h/mg)	0.03	0.028 (0.020 – 0.039)
	Vmax, kidney (µmol/h/mg)	0.004	NA
	Km, liver (µmol/L)	0.88	0.63 (0.44 – 0.90)
	Km, lung (µmol/L)	2.82	2.95 (2.04 – 4.31)
	Km, kidney (µmol/L)	176.11	NA
	KF, kidney (L/h/g)	NA	0.42 (0.17 – 0.73)
Male mouse	Vmax, liver (µmol/h/mg)	0.26	0.25 (0.24 – 0.26)
	Vmax, lung (µmol/h/mg)	0.14	0.14 (0.12 – 0.15)
	Vmax, kidney (µmol/h/mg)	0.01	0.010 (0.008 – 0.014)
	Km, liver (µmol/L)	1.34	0.99 (0.89 – 1.09)
	Km, lung (µmol/L)	2.22	1.99 (1.64 – 2.29)
	Km, kidney (µmol/L)	0.77	0.61 (0.43 – 0.85)
	KF, lung (L/h/g)	NA	NA
Female rat	Vmax, liver (µmol/h/mg)	0.09	0.074 (0.059 – 0.092)
	Vmax, lung (µmol/h/mg)	NA	NA
	Vmax, kidney (µmol/h/mg)	0.003	0.0036 (0.0028 – 0.0046)
	Km, liver (µmol/L)	0.56	0.87 (0.66 – 1.14)
	Km, lung (µmol/L)	NA	NA
	Km, kidney (µmol/L)	0.60	0.57 (0.42 – 0.78)

	KF, lung (L/h/g)	0.96	0.12 (0.048 – 0.49)
Male rat	Vmax, liver (µmol/h/mg)	0.10	0.074 (0.071 – 0.077)
	Vmax, lung (µmol/h/mg)	NA	NA
	Vmax, kidney (µmol/h/mg)	0.003	0.0040 (0.0031 – 0.0050)
	Km, liver (µmol/L)	0.56	0.46 (0.42 – 0.49)
	Km, lung (µmol/L)	NA	NA
	Km, kidney (µmol/L)	0.76	0.84 (0.63 – 1.08)
	KF, lung (L/h/g)	1.28	0.25 (0.05 – 0.49)
Humans	Vmax, liver (µmol/h/mg)	0.05	0.053 (0.052 – 0.055)
	Vmax, lung (µmol/h/mg)	NA	NA
	Vmax, kidney (µmol/h/mg)	NA	NA
	Km, liver (µmol/L)	0.45	0.38 (0.36 – 0.41)
	Km, lung (µmol/L)	NA	NA
	Km, kidney (µmol/L)	NA	NA
	KF, lung (L/h/g)	0.32	1.5E-11 (7.5 E-23 – 0.44)

Table S-4: Metabolism Parameters for PBPK Model

Sex & Species	Parameter	Source	
		Yang et al. 2012	This Analysis (Table S-3)
Female mouse	VmaxC, liver (mg/h/kg**3/4)	8.88	8.33
	VmaxC, lung (mg/h/kg**3/4)	0.11	0.18
	VmaxC, kidney (mg/h/kg**3/4)	0.03	NA
	Km, liver (mg /L)	0.08	0.055
	Km, lung (mg /L)	0.25	0.26
	KM, kidney (mg /L)	9.59	NA
	KF, kidney (L/h/kg)	NA	0.077
Male mouse	VmaxC, liver (mg/h/kg**3/4)	18.54	18.99
	VmaxC, lung (mg/h/kg**3/4)	0.6	0.91
	VmaxC, kidney (mg/h/kg**3/4)	0.078	0.074
	Km, liver (mg /L)	0.12	0.088
	Km, lung (mg /L)	0.2	0.18
	KM, kidney (mg /L)	0.068	0.054
	KF, lung (L/h/kg)	NA	NA
Female rat	VmaxC, liver (mg/h/kg**3/4)	9.37	7.30
	VmaxC, lung (mg/h/kg**3/4)	NA	NA
	VmaxC, kidney (mg/h/kg**3/4)	0.018	0.019
	Km, liver (mg /L)	0.09	0.077
	Km, lung (mg /L)	NA	NA
	KM, kidney (mg /L)	0.053	0.050

	KF, lung (L/h/kg)	0.16	0.084
Male rat	VmaxC, liver (mg/h/kg**3/4)	9.48	7.87
	VmaxC, lung (mg/h/kg**3/4)	NA	NA
	VmaxC, kidney (mg/h/kg**3/4)	0.018	0.024
	Km, liver (mg /L)	0.05	0.040
	Km, lung (mg /L)	NA	NA
	KM, kidney (mg /L)	0.067	0.075
	KF, lung (L/h/kg)	0.15	0.092
Humans	VmaxC, liver (mg/h/kg**3/4)	20.4	17.53
	VmaxC, lung (mg/h/kg**3/4)	NA	0.0034
	VmaxC, kidney (mg/h/kg**3/4)	0	0
	Km, liver (mg /L)	0.04	0.034
	Km, lung (mg /L)	NA	0.034
	KM, kidney (mg /L)	NA	NA
	KF, lung (L/h/kg)	0.05	NA

Table S-5: Plethysmography Data

Pulmonary Function Data Protocol 07039 Summary			Exposure #1
Exposure	**Frequency (BPM)**	**Tidal Volume (ml)**	**Minute Ventilation (ml/min)**
0ppm	Average	Average	Average
Animal 1	193.5	0.247	48.4
Animal 2	168.2	0.284	47.2
Animal 3	251.9	0.212	51.6
Animal 4	201.7	0.311	61.6
Average	203.8	0.264	52.2
Stdev	35.1	0.043	6.5

Pulmonary Function Data Protocol 07039 Summary			Exposure #2
Exposure	**Frequency (BPM)**	**Tidal Volume (ml)**	**Minute Ventilation (ml/min)**
13 ppm	Average	Average	Average
Animal 20	221.9	0.261	57.6
Animal 21	221.0	0.264	57.6
Animal 22	140.9	0.224	32.0
Animal 23	131.2	0.218	28.9
Average	178.7	0.242	44.0
Stdev	49.4	0.024	15.7

Pulmonary Function Data Protocol 07039 Summary			Exposure #3
Exposure **32 ppm**	**Frequency (BPM)** Average	**Tidal Volume (ml)** Average	**Minute Ventilation (ml/min)** Average
Animal 54	253.3	0.269	66.1
Animal 55	225.0	0.269	59.4
Animal 56	249.8	0.282	69.5
Animal 57	235.2	0.293	67.9
Average	240.8	0.278	65.7
Stdev	13.1	0.012	4.4

Pulmonary Function Data Protocol 07039 Summary			Exposure #4
Exposure **90 ppm**	**Frequency (BPM)** Average	**Tidal Volume (ml)** Average	**Minute Ventilation (ml/min)** Average
Animal 88	216.3	0.276	59.4
Animal 89	178.4	0.242	43.2
Animal 90	217.9	0.277	59.8
Animal 91	166.2	0.278	45.8
Average	194.7	0.268	52.0
Stdev	26.4	0.017	8.8

Table S-5: Arterial Blood Concentrations

13 ppm Exposure			
Time point	**Average [CD]**	**Std Dev. CD**	**RSD%**
Control	0	0	0%
0.5 hours	1.03	0.18	17%
3 hours	1.93	0.80	41%
6 hours	1.58	0.35	22%
5 min post exposure	0.66	0.07	101%
10 min post exposure	0.70	0.11	157%
15 min post exposure	0	0	0%
32 ppm Exposure			
Time point	**Average [CD]**	**Std Dev. CD**	**RSD%**
Control	0	0	0%
0.5 hours	1.68	0.70	42%
3 hours	2.90	1.15	40%
6 hours	2.44	1.24	51%
5 min post exposure	0.61	0.22	35%
10 min post exposure	0.18	0.09	48%
15 min post exposure	0.30	0.23	78%
90 ppm Exposure			
Time point	**Average [CD]**	**Std Dev. CD**	**RSD%**
Control	0	0	0%

0.5 hours	6.41	1.83	29%
3 hours	7.33	3.52	48%
6 hours	8.00	1.02	13%
5 min post exposure	1.71	0.78	46%
10 min post exposure	0.92	0.33	35%
15 min post exposure	0.68	0.15	23%

C. Re-estimation of Metabolism Parameters

The original *in vitro* concentration time-course data for metabolism of chloroprene (Himmelstein et al. 2004a, IISRP 2009b) were re-analyzed using MCMC analysis with vague priors to obtain a revised set of metabolism parameters for the model. The key differences between the alternative analysis and the original Yang et al. (2012) analysis were: (1) the incorporation of an additional parameter in the analysis of the *in vitro* metabolism data (Kg) to describe the rate of transfer of chloroprene from the headspace to the media in the metabolism studies, (2) the use of updated tissue microsomal protein concentrations for scaling the *in vitro* results to *in vivo* values appropriate for the PBPK model, and (3) the adoption of a previously published approach for estimating the metabolism parameters in the human lung (Andersen et al. 1987). The details of the re-estimation of the in vitro metabolism parameters is described below and the results are provided in Supplemental Materials F.

Experimental Determination of Mass Transport Limitation

Schlosser et al. (1993) have suggested the need to consider mass transport limitations during i*n vitro metabolism* experiments conducted with volatile compounds where there is an air:liquid interface. In their studies of benzene metabolism in sealed vials (Schlosser et al. 1993), they conducted separate studies to determine the rate of approach of the system to equilibrium in order to estimate the rate of transfer of the chemical between the air and liquid phases, and then used the estimated mass transport parameter (Kg) in their analysis of the metabolism of benzene. In the *in vitro* metabolism studies conducted with chloroprene (Himmelstein et al. 2004a, Yang et al. (2012), no assessment of mass transport limitation was performed. Therefore, a new experimental study was performed to estimate a Kg for chloroprene following a protocol similar to Schlosser et al. (1993). The study report is provided in Supplemental Materials D. The protocol for the study is reproduced below:

1. Add 1mL buffer solution to a 10mL crimp top vial. Crimp on top. A total of 12 vials will be needed for each series of tests.
2. Place vials in a water bath set at 37 C rotating at 60 rpm. Allow temperature to equilibrate for a minimum of 10 minutes.
3. After reaching thermal equilibrium, pierce the septa with an open needle to allow the pressure of the headspace to adjust to ambient pressure.
4. Add 0.5mL of 800ppmv chloroprene gas standard*. Immediately start a timer set for the appropriate contact time.

5. As the timer reaches zero, remove vial and insert a 1mL syringe into the vial so the needle is below the liquid level in the vial. Withdraw 0.5mL and place it into a 40mL VOA vial containing a Teflon stir bar and 4.5mL of deionized water.
6. The beginning and ending blanks are treated the same as the samples with the addition of the chloroprene.
7. A total of 10 samples are to be prepared with contact times of 5, 10, 20, 30, 45, 60, 120, 180, 240and 360 seconds. The 5 second sample was replaced with a 600 second sample starting with replicate set R-15.
8. When all of the samples for the set are prepared in the 40mL VOA vials, they are analyzed in the VOA lab by GC\MS SW-846 Method 8260B and Method 5035. The concentration of the chloroprene is reported in ug/L.

*The vapor standard used to spike the headspace was prepared according to Denka Method PW R Gas 1805 using a chloroprene standard received from Denka. A high concentration stock was prepared and a working standard prepared from the stock by doing a 100x dilution into a second Tedlar bag. The initial vapor standards were prepared in 1L Tedlar bags. The remaining standard sets were prepared in 500mL Tedlar bags.

The resulting time-courses for chloroprene concentrations in the aqueous phase of the vials were analyzed using the same approach as in Schlosser et al. (1993), resulting in an estimated value of 0.024 L/hr for Kg, which is similar to the value previously reported for benzene (Schlosser et al. 1993). The results of the analysis are shown in Figure B-1.

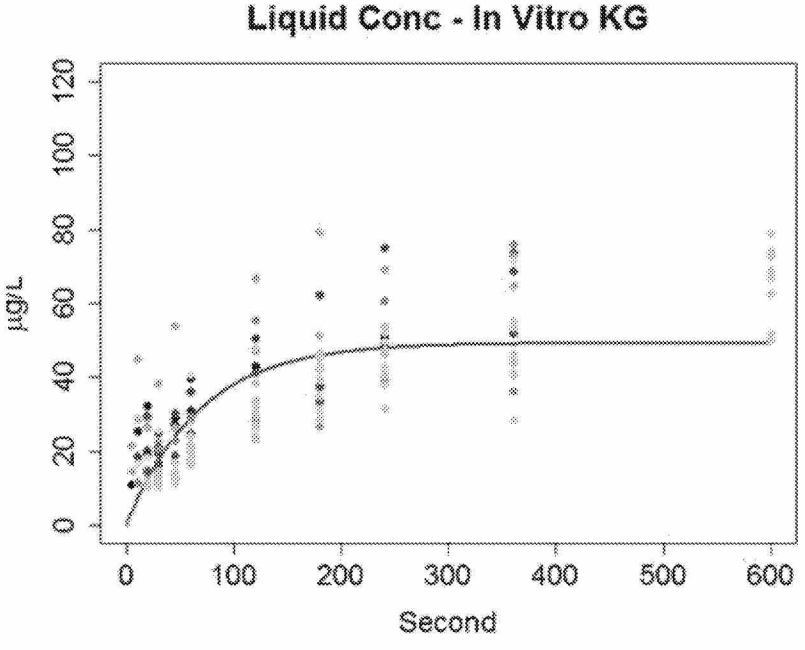

Figure B-1. Concentration of chloroprene in the aqueous phase following addition of 0.5 mL of 800 ppmV chloroprene to the air phase. The best estimates of Kg and P (the liquid:air partition coefficient) were 0.024 (std. dev. = 0.0054) and 0.48 (std. dev. = 0.02).

Using this experimental value of Kg, however, it was not possible to explain the high rates of liver metabolism observed at low concentrations of chloroprene; that is, the mass transport associated with Kg = 0.024 L/hr was too slow to support the observed rates of metabolism in the media. It is possible that the presence of microsomes during the metabolism studies resulted in faster uptake than in the Kg study by competing with non-specific surface binding of chloroprene, which is highly lipophilic. No microsomes were present in the benzene Kg experiments (Schlosser et al. 1993) either.

Re-estimation of *In Vitro* Metabolism Parameters

As an alternative approach for determining the Kg in the original metabolism studies, a re-analysis of the *in vitro* data was performed attempting to simultaneously estimate Kg along with the metabolism parameters, Vmax and Km. However, a high degree of collinearity between Km and Kg made it impossible to estimate the parameters independently. To resolve this situation, we conducted a literature review of compounds with structural similarities to chloroprene, specifically, halogenated alkanes and alkenes, and found that the values of Km estimated for these compounds from a variety of *in vivo* studies in mice, rats and humans ranged from 1 to 5 µmol/L (Andersen et al. 1987b,1991, 1994; Clewell et al. 2001; Corley et al. 1990; David et al. 2006; D'Souza et al. 1987, 1988; D'Souza and Andersen 1988; Gargas et al. 1986, 1990; Gargas and Andersen 1989; Lilly et al. 1997, 1998; Marino et al. 2006). The strongest data were from studies of mutual metabolic inhibition in co-exposures to trichloroethylene and dichloroethylene (Andersen et al. 1987b), which estimated Kms of 1.9 and 1.0 µmol/L, respectively. A Km of 1.6 µmol/L was used by the USEPA in their risk assessment for vinyl chloride (USEPA 2000). Therefore, a re-analysis of the data on metabolism in the male mouse liver was performed to estimate Vmax and Kg with Km set to a fixed value of value of 1.0 µmol/L. The resulting value of Kg (0.46 L/hr) was then used in the MCMC analysis of the metabolism data for all tissues. The details of the MCMC analysis are provided below.

Yang et al. (2012) reported a two-level hierarchical Bayesian model to estimate the gender-variability of the in vitro metabolic parameters. In the re-analysis of the in vitro data presented here, the primary interest was on point estimates of metabolic constants for each species and sex (mixed gender for human) in the presence of our predicted flux of chloroprene between air and media in the in vitro system. The single level analysis retained the broad prior distributions used in the Yang analysis. Parameters for both the saturable (i.e., Vmax and Km) and the 1st order (Vk, where Km was unidentifiable) metabolism of chloroprene were given broad log-uniform distributions (-10, 5) and the broad likelihood (lognormal, 0.3, 5) was used to avoid over-constraining the posterior parameters. The flux of chloroprene between air and media (Kg) was estimated by fixing the Km in the male mouse liver microsomal study to 1.0 μmol/L and estimating both Vmax and Kg. Initial testing of the model showed that the male mouse liver had the strongest data upon which to base the Kg (i.e., steepest slope as low start concentrations). In the estimation of Kg, the broad distributions reported above for metabolic parameters were retained. The geometric mean of Kg was retained as a fixed value for the analysis of all the in vitro studies including the male mouse liver which was re-analyzed to estimate Vmax and Km after the Kg was fixed. In one case, mixed human lung, it was noted that the lower truncation limit for Vk was skewing the posterior distribution of the inherent uncertainty in the 1st order process in the human. For the mixed human lung simulation, the lower truncation was reduced to -60 to determine if a lower bound could be found. In one additional instance, female mouse kidney, the Km was not identifiable from the in vitro study and the analysis was used to estimate a 1st order metabolic rate constant. The posterior distribution indicates that this is not the case (data now shown) and the upper 95th percentile of this posterior distribution had been represented as the mean in Yang et al. (2012). For Vmax and Km analysis, 20000 iterations of the model were run with the first 10000 discarded for the posterior analysis and for the Vk analysis, 10000 iterations of the model were run with the final 6000 retained for posterior analysis.

For all analysis of the in vitro data with MCMC, three chains were run independently with different start values to test stability of model convergence. The truncated chains were assessed for convergence both visually (line and density plots) and by verifying that the corrected scale reduction factor was less than 1.2. The results of the corrected scale reduction factor analysis (exported from R) and the posterior distributions for the parameters and the likelihood are listed

in the supplemental Excel workbook (Supplemental Materials F) under the species-specific worksheets.

Example Posterior Distributions

Examples of the posterior distributions from the MCMC analysis are presented below:

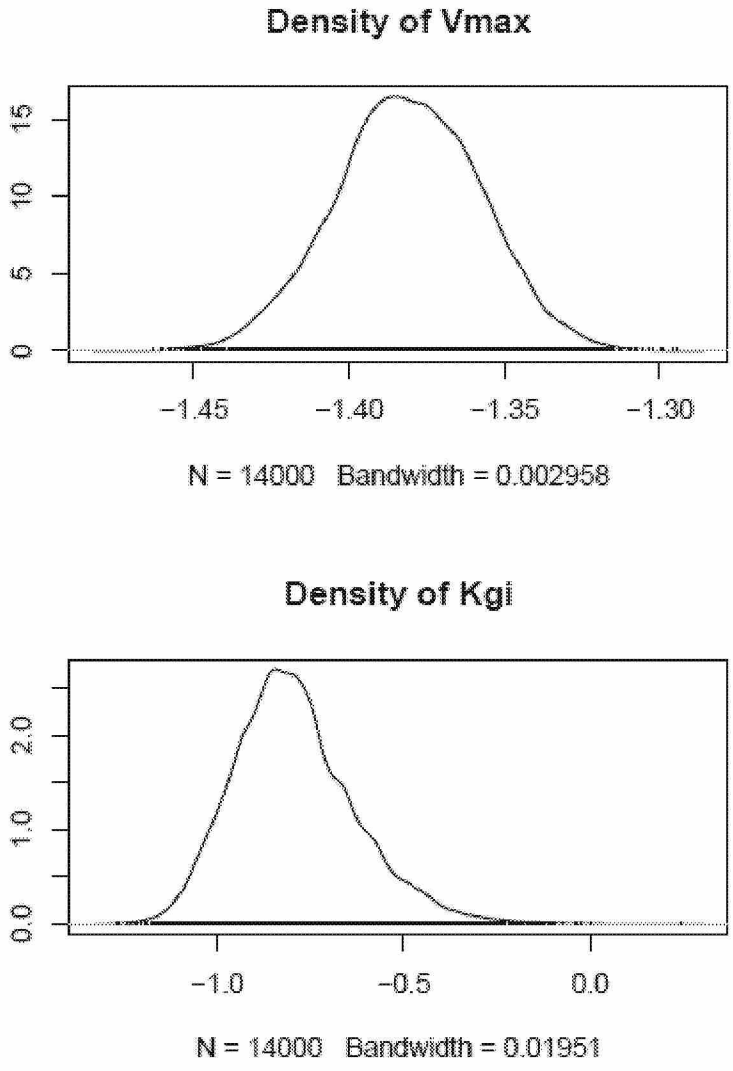

Figure C-1. Posterior distributions for ln(Vmax) (top) and ln(Kg) (bottom). Km was fixed at 1.0 mg/L for this analysis.

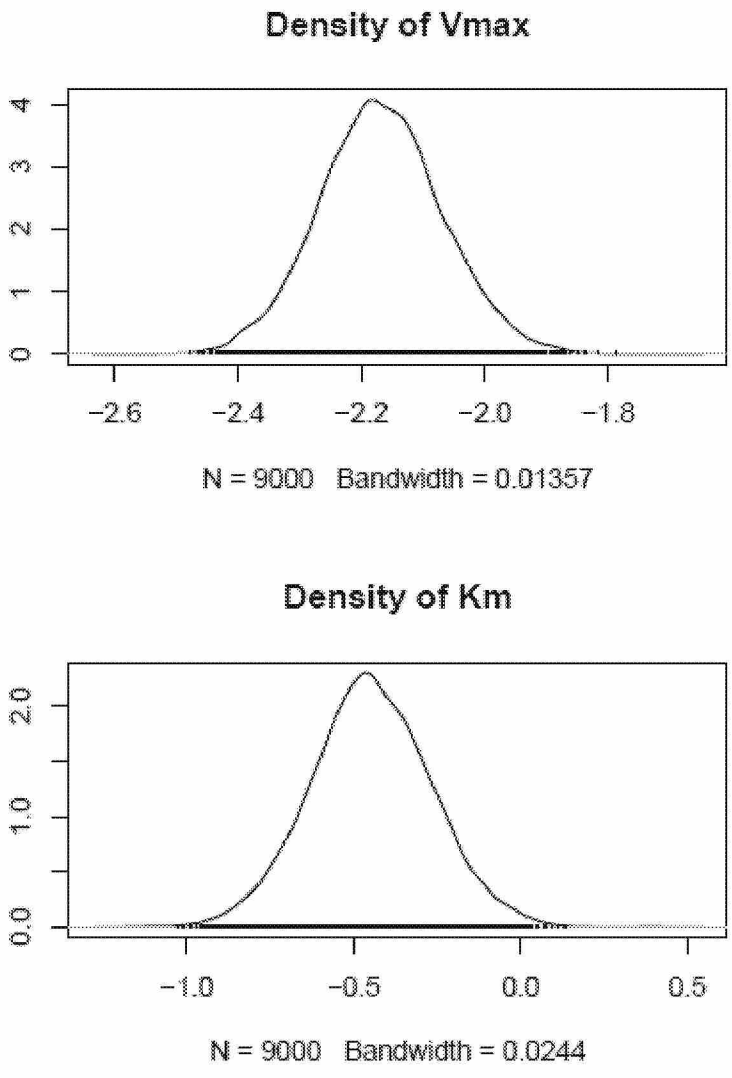

Figure C-2. Posterior distributions for ln(Vmax) (top) and ln(Km) (bottom) in female mouse liver. Kg was fixed at 0.45 L/hr for this analysis.